

Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1. (original) A method of labelling a nucleic acid, wherein the method comprises incorporating at least one nucleotide analog into a nucleic acid; said analog comprising an 8-S-substituted purine or 5-S-substituted pyrimidine analog of the formula NucSR;

wherein Nuc is pyrimidinyl or purinyl;

wherein S is sulfur;

wherein R is selected from the group consisting of:

H, a hapten, biotin, an enzyme, a protein, an abortive promoter cassette, a photocrosslinker, a chemical crosslinker, streptavidin, a fluorescent moiety, a colorimetric moiety, a luminescent moiety, a chemiluminescent moiety, a metal, a dye, a nucleic acid cellular uptake group, a C₆₋₁₀ aryl, C₆₋₁₀ ar(C₁₋₆)alkyl, C₆₋₁₀ arylamino(C₁₋₆)alkyl, C₆₋₁₀ aryloxy(C₁₋₆)alkyl, C₆₋₁₀ ar(C₁₋₆)alkylamino(C₁₋₆)alkyl, C₆₋₁₀ ar(C₁₋₆)alkyloxy(C₁₋₆)alkyl, C₆₋₁₀ ar(C₁₋₆ alkyl)carbonylamino(C₁₋₆)alkyl, C₆₋₁₀ ar(C₁₋₆ alkyl)carbonyloxy(C₁₋₆)alkyl, (C₆₋₁₀ aryl)carbonylamino(C₁₋₆)alkyl, (C₆₋₁₀ aryl)carbonyloxy(C₁₋₆)alkyl, (C₆₋₁₀ aryl)carbonyl(C₁₋₆)alkyl and C₆₋₁₀ ar(C₁₋₆ alkyl)carbonyl(C₁₋₆)alkyl, wherein the aryl portion of each of the preceding groups is optionally substituted with 1-4 substituents independently selected from the group consisting of halo, hydroxyl, C₁₋₆ alkyl, C₃₋₈ cycloalkyl, C₁₋₆ haloalkyl, C₁₋₆ hydroxyalkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkoxy, (C₁₋₆ alkyl)carbonyl, (C₁₋₆ alkoxy)carbonyl, amino, amino(C₁₋₆)alkyl, aminocarbonyl, mono(C₁₋₆ alkyl)aminocarbonyl, di(C₁₋₆ alkyl)aminocarbonyl, C₁₋₆ alkylamino, di(C₁₋₆)alkylamino, (C₁₋₆ alkyl)carbonylamino, C₆₋₁₀ arylamino, (C₆₋₁₀ aryl)carbonylamino, mono(C₆₋₁₀ aryl)aminocarbonyl, di(C₆₋₁₀ aryl)aminocarbonyl, mono(C₆₋₁₀ ar(C₁₋₆ alkyl))aminocarbonyl, di(C₆₋₁₀ ar(C₁₋₆ alkyl))aminocarbonyl, N-(C₆₋₁₀)aryl-N-(C₁₋₆ alkyl)aminocarbonyl, N-(C₆₋₁₀)ar(C₁₋₆)alkyl-N-(C₁₋₆ alkyl)aminocarbonyl, N-(C₆₋₁₀)ar(C₁₋₆)alkyl-N-(C₆₋₁₀ aryl)aminocarbonyl, C₁₋₆ alkylthio,

C₆₋₁₀ arylthio, C₆₋₁₀ ar(C₁₋₆)alkylthio, carboxy, carboxy(C₁₋₆)alkyl, nitro, cyano, heteroaryl and saturated or partially unsaturated heterocycle, wherein the heteroaryl and saturated or partially unsaturated heterocycle are independently monocyclic or fused bicyclic and independently have 5 to 10 ring atoms, wherein one or more of the ring atoms are independently selected from the group consisting of oxygen, nitrogen and sulfur; *provided that* (1) when the aryl portion of R is substituted with two substituents, at least one of the substituents is other than nitro, and (2) when R is phenylacetamidomethyl, the phenyl portion is substituted at least once.

2. (original) The method of claim 1, wherein R is selected from the group consisting of:

a hapten, biotin, an enzyme (e.g horseradish peroxidase or alkaline phosphatase), a protein, an abortive promoter cassette, a photocrosslinker, a chemical crosslinker, streptavidin, a fluorescent moiety, a colorimetric moiety, a luminescent moiety, a chemiluminescent moiety, a metal, a dye, and a nucleic acid cellular uptake group.

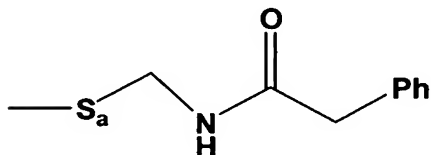
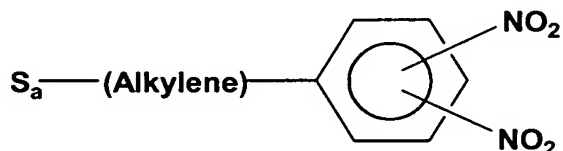
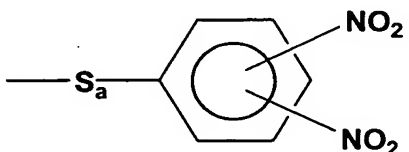
3. (original) The method of claim 1, wherein R is selected from the group consisting of:

H, phenyl, phenyl(C₁₋₆)alkyl, phenylamino(C₁₋₆)alkyl, phenoxy(C₁₋₆)alkyl, phenyl(C₁₋₆)alkylamino(C₁₋₆)alkyl, phenyl(C₁₋₆)alkyloxy (C₁₋₆)alkyl, phenyl(C₁₋₆ alkyl)carbonylamino(C₁₋₆)alkyl, phenyl(C₁₋₆ alkyl)carbonyloxy(C₁₋₆)alkyl, benzoylcarbonylamino(C₁₋₆)alkyl, benzoyloxy (C₁₋₆)alkyl, benzoyl(C₁₋₆)alkyl and phenyl(C₁₋₆ alkyl)carbonyl(C₁₋₆)alkyl, wherein the aryl portion of each of the preceding groups is optionally substituted with 1-2 substituents independently selected from the group consisting of halo, hydroxyl, C₁₋₆ alkyl, C₃₋₈ cycloalkyl, C₁₋₆ haloalkyl, C₁₋₆ hydroxyalkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkoxy, (C₁₋₆ alkyl)carbonyl, (C₁₋₆ alkoxy)carbonyl, amino, amino(C₁₋₆)alkyl, aminocarbonyl, mono(C₁₋₆ alkyl)aminocarbonyl, di(C₁₋₆ alkyl)aminocarbonyl, C₁₋₆ alkylamino, di(C₁₋₆) alkylamino, (C₁₋₆ alkyl)carbonylamino, phenylamino, benzoylamino, phenylaminocarbonyl, diphenylaminocarbonyl, phenyl(C₁₋₆ alkyl) aminocarbonyl, di(phenyl(C₁₋₆

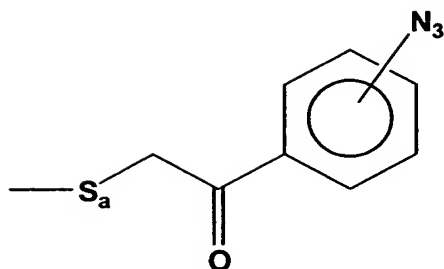
alkyl))aminocarbonyl, *N*-phenyl-*N*-(C₁₋₆ alkyl) aminocarbonyl, *N*-phenyl(C₁₋₆)alkyl-*N*-(C₁₋₆ alkyl)aminocarbonyl, *N*-phenyl (C₁₋₆)alkyl-*N*-phenylaminocarbonyl, C₁₋₆ alkylthio, phenylthio, phenyl(C₁₋₆) alkylthio, carboxy, carboxy(C₁₋₆)alkyl, nitro and cyano.

4. (original) The method of claim 1, wherein R is selected from the group consisting of:
 H, dinitrophenol, and APAS.

5. (original) The method of claim 1, wherein SR is selected from the group consisting of:



and



wherein a is 1 or 2.

6. (original) The method of claim 1, wherein the nucleotide analog is incorporated into nucleic acid by an enzymatically mediated process, the process selected from the group consisting of:

- DNA-dependent DNA polymerization;
- RNA-dependent DNA polymerization;
- DNA-dependent RNA polymerization; and
- RNA-dependent RNA polymerization;

7. (original) The method according to claim 1, wherein said analog is incorporated into a nucleic acid by a method selecting from the group consisting of

- polymerase chain reaction;
- nick translation;
- reverse transcription;
- terminal transferase addition;
- ligation;
- transcription; and
- abortive transcription.

8. (original) The method of claim 1, wherein said analog is incorporated into a nucleic acid via chemical coupling.

9. (original) A method of detecting a second nucleic acid of interest, said method involving

a) producing a first molecule of nucleic acid; wherein said first molecule is produced by the method of claim 1;

mixing said first nucleic acid with a second nucleic acid such that said first and second nucleic acids are associated by Watson Crick base pairing;
detecting the presence of the first nucleic acid.

10. (original) The method of claim 9, wherein said method is selected from the group consisting of:

Southern blotting;
Northern blotting; and
Microarray hybridization.

11. (original) A method for detecting multiple reiterated oligonucleotides from a target DNA or RNA polynucleotide, said method comprising:

(a) hybridizing an initiator with a single stranded target polynucleotide;

(b) incubating said target polynucleotide and initiator with an RNA-polymerase, and a terminator;

(c) synthesizing multiple oligonucleotides from said target polynucleotide, wherein said initiator is extended until said terminator is incorporated into said oligonucleotides thereby synthesizing multiple reiterative oligonucleotides; and
detecting or quantifying said reiteratively synthesized oligonucleotide transcripts of a polynucleotide of interest by incorporating a modified nucleotide analog in at least one of the members selected from the group consisting of said terminator, and said initiator.

Claims 12-15 (cancelled)

16. (original) The method of claim 11, wherein said chain terminator comprises a nucleotide analog.

17. (original) A method of detecting multiple reiterated oligonucleotides from a target DNA or RNA polynucleotide, said method comprising:

- (a) hybridizing an initiator to a single-stranded target polynucleotide;
- (b) incubating said target polynucleotide and initiator with a target site probe, an RNA-polymerase, and a terminator, wherein said target site probe hybridizes with said target polynucleotide;
- (c) synthesizing an oligonucleotide transcript that is complementary to said target site from said target polynucleotide, wherein said initiator is extended until said terminator is incorporated into said oligonucleotide transcript, thereby synthesizing multiple reiterative oligonucleotide transcripts; and
- (d) detecting or quantifying said reiteratively synthesized oligonucleotide transcripts to about 100 nucleotides and greater than 100 nucleotides by incorporating a nucleotide analog into at least one of the members selected from the group consisting of said terminator, said initiator, and said target-site probe.

18. (original) A method for detecting methylated cytosine residues at CpG sites in a target polynucleotide, comprising:

- (a) deaminating a single-stranded target DNA sequence under conditions which convert unmethylated cytosine residues to uracil residues while not converting methylated cytosine residues to uracil;
- (b) hybridizing an initiator with a single stranded target polynucleotide;
- (c) incubating said deaminated target polynucleotide and said initiator with a terminator, and an RNA-polymerase, wherein at least one of said initiator, or terminator is modified to enable detection of the CG sites;
- (d) synthesizing an oligonucleotide transcript that is complementary to said CG sites from said target polynucleotide, wherein said initiator is extended until said terminator is incorporated into said oligonucleotide transcript thereby synthesizing multiple reiterative oligonucleotide transcripts;

(e) detecting or quantifying said reiteratively synthesized oligonucleotide transcripts by incorporating a nucleotide analog into at least one of the members selected from the group consisting of said terminator, and said initiator.

19. (original) A method for detecting the presence or absence of mutations in a target DNA sequence, the method comprising

(a) hybridizing a target site probe to a single-stranded DNA polynucleotide, wherein said DNA polynucleotide may contain a mutation relative to a normal or wild type gene;

(b) incubating said target polynucleotide and target-site probe with an RNA-polymerase, an initiator, and a terminator;

(c) synthesizing an oligonucleotide transcript from said target polynucleotide that is complementary to a target mutation site, wherein said initiator is extended until said terminator is incorporated into said oligonucleotides thereby synthesizing multiple abortive reiterative oligonucleotides; and

(d) determining the presence or absence of a mutation by detecting or quantifying said reiteratively synthesized oligonucleotides transcribed from said target DNA polynucleotide by incorporating a nucleotide analog into at least one of the members selected from the group consisting of said terminator, and said initiator.

20. (original) A method for detecting mutations in a target DNA polynucleotide, said method comprising:

(a) immobilizing a capture probe designed to hybridize with said target DNA polynucleotide;

(b) hybridizing said capture probe to said target DNA polynucleotide, wherein said DNA polynucleotide may contain a mutation relative to a normal or wild type gene;

(c) incubating said target polynucleotide with an RNA-polymerase, an initiator, and a terminator;

(d) synthesizing an oligonucleotide transcript that is complementary to a target site from said target polynucleotide, wherein said initiator is extended until said terminator is incorporated into said oligonucleotide transcript, thereby synthesizing multiple abortive reiterative oligonucleotide transcripts;

(e) determining the presence or absence of a mutation by detecting or quantifying said reiteratively synthesized oligonucleotide transcripts from said target DNA polynucleotide by incorporating a nucleotide analog into at least one of the members selected from the group consisting of said terminator, and said initiator.

21. (original) A method for detecting DNA or RNA in a test sample, said method comprising:

(a) hybridizing a single stranded target polynucleotide with an artificial promoter cassette comprising a sequence that hybridizes to the single stranded target polynucleotide, and a region that can be detected by transcription by a polymerase;

(b) incubating said target polynucleotide with an RNA-polymerase, an initiator, and a terminator;

(c) synthesizing an oligonucleotide transcript that is complementary to the initiation start site of the APC, wherein said initiator is extended until said terminator is incorporated into said oligonucleotides, thereby synthesizing multiple reiterative oligonucleotide transcripts; and

(d) detecting or quantifying said reiteratively synthesized oligonucleotide transcripts by incorporating a nucleotide analog into at least one of the members selected from the group consisting of said terminator, and said initiator.

22. (original) A method for detecting the presence of pathogens in a test sample, said method comprising:

(a) hybridizing a single stranded target pathogen polynucleotide in said test sample with an artificial promoter cassette comprising a region that can be detected by transcription by a polymerase;

(b) incubating said target polynucleotide and initiator with an RNA-polymerase, and a terminator;

(c) synthesizing an oligonucleotide transcript that is complementary to initiation start site of the APC, wherein said initiator is extended until said terminator is incorporated into said oligonucleotides thereby synthesizing multiple abortive reiterative oligonucleotide transcripts; and

(d) determining the presence of a pathogen by detecting or quantifying said reiteratively synthesized oligonucleotide transcripts synthesized from said test sample by incorporating a nucleotide analog into at least one of the members selected from the group consisting of said terminator, and said initiator.

23. (original) A method for detecting mRNA expression in a test sample, the method comprising:

(a) hybridizing a target mRNA sequence with an artificial promoter cassette comprising a region that can be detected by transcription by a polymerase;

(b) incubating said target mRNA sequence with an RNA-polymerase, an initiator, and a terminator;

(c) synthesizing an oligonucleotide transcript that is complementary to transcription initiation start site, wherein said initiator is extended until said terminator is incorporated into said oligonucleotide transcript, thereby synthesizing multiple reiterative oligonucleotides; and

(d) determining the presence or absence of the mRNA by detecting or quantifying said reiteratively synthesized oligonucleotide transcripts synthesized from said test sample by incorporating a nucleotide analog into at least one of the members selected from the group consisting of said terminator, and said initiator.

24. (original) A method for detecting an oligonucleotide synthesized from a target DNA sequence, the method comprising:

(a) hybridizing a DNA primer with a single-stranded target DNA sequence;

(b) extending said DNA primer with a DNA polymerase and nucleotides, such that said DNA polymerase reiteratively synthesizes a nucleotide sequence; and

(c) detecting oligonucleotide comprised of repeat sequences synthesized by said DNA polymerase by incorporating a nucleotide analog to enable detection of said oligonucleotide comprised of repeat sequences.

Claims 25-27 (cancelled)